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STUDIES ON THE GASTRIC JUICE PROTEIN

PART II PAPER ELECTROPHORESIS-POLAROGRAPHIC STUDY OF GARTRIC JUICE PROTEIN

by

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INTRODUCTION

The complexity of the protein component of gastric juice has been recognized only recently. The most convincing evidence of its complexity was afforded by the study of the electrophoretic patterns and fractionation of the juice collected from several dogs by Grossberg, Komarov and Shay.[1] Since then electrophoretic studies of gastric juice have been reported by many investigators.[2)~9)] A charcteristic change in peptide and protein contents of gastric juice with gastric cancer, using polarographic and fractional precipitation methods was noted in a previous report.[10)]

This paper describes my further efforts to clarify the origin, nature and clinical significance of the gastric peptide and protein, applying a combined new method of paper electrophoresis and polarography.

I. FUNDAMENTALS

MATERIALS

A) 10 acid gastric juice specimens obtained from 10 normal cases.

B) 10 anacid gastric juice specimens obtained from 10 cases with achlorhydria without gastric cancer.

C) 10 dialysed gastric juice specimens prepared as follows; 10 acid gastric juice (material A) were dialysed through a cellophane membrane against frequently exchanged cold distilled water in a refrigerator for 24 hours.

D) 10 dialysates of the acid gastric juice (material A).

E) Methanol filtrate prepared from acid gastric juice (material A) as described previously.[10)]

F) Blood serum of a healthy person.

G) Pepsin digested serum obtained as follows:
Serum was adjusted to pH 2.0 with 0.1-N HCl solution, mixed with crystal pepsin at 10^{-3} concentration and incubated at 37°C for 15 minutes, centrifuged for 15 minutes at 3000 rpm.

H) Pepsin digested anacid juice prepared as described above.

I) B-bile aspirated through a duodenal tube from a healthy person.

J) Gastric juice collected from 2 Pavlov's pouch dogs two and four weeks after operation.

Only juices not contaminated with bile, blood or food were used, the contamination with saliva was minimized by strict precautions taken during collection of the juice.

METHODS

The samples of gastric juice were subsequently filtered through a filter paper (Toyo Roshi No. 5c). The materials other than serum were neutralized with 1N-NaOH and freeze-dried by lyophilization.

Electrophoresis: Lyophilized materials were dissolved at 5% concentration in a borate buffer at pH 9.0 and ionic strength of 0.12 (prepared by dissolving 15.3 g $Na_2B_4O_7$ and 1.24 g H_3BO_3 in 1000ml of distilled water as described by GLASS)[11]. Each 0.05 ml of this solution containing 2.5 mg of dry material or 0.05 ml of serum were applied to the center of each of the Toyo Roshi No. 51 paper strips, 3 cm wide, mounted in one cell of Toyo Roshi Co., and electrophoresis was carried out against the same borate buffer at 0.45 ma. per cm and 5 volts per cm, for 8 hours at room temperature. Immediately after electrophoresis five strips were removed from the cell, and 3 out of 5 strips were oven-dried for 30 minutes at 100°C. The first strip was stained with brom phenol blue stain[12] (abbreviated as BPB stain hereafter), the second strip with periodic Shiff stain[12] (PAS stain), the third strip was sprayed with 0.2% ninhydrine in butanol saturated with acetate buffer of pH 5.0 after spraying with the same acetate buffer and heated to 90°C for 15 minutes.[13] All stained strips were then scanned and traced in a densitometer (Toyo Roshi Co.), using a 2 mm slit.

Elution: The remaining 2 strips were not dried or stain, but were cut into 16 segments, 10 mm wide, from 8 cm on the cathodic side of the application point to 8 cm on the anodic side, numbered from C8 to A8, respectively. 2 segments corresponding to each number were put together into one jar of the same number. 2 ml of NaCl solution or 2 ml of 10% sulfosalicylic acid was added to each of the 16 jars. These were placed in a refrigerator for 12 hours.

Polarograph:[14][15][16] 16 electrolysis cells were numbered from C8 to A8, cleansed and dried thoroughly. Into each cell was poured 1.0 ml of trivalent cobalt test solution which was prepared as follows:

A) 2-N ammonium chloride solution

B) 2×10^{-3}N hexaminic cobaltic chloride (luteosalt) solution

C) 2-N ammonium hydroxide solution

The test solution was prepared by adding just before the experiments in this order; 1 vol. of A, 1 vol. of B, 8 vol. of C. To each cell of the same number was added 1.0 ml of each of the 16 eluates. Polarograms were taken immediately in each cell under the same conditions as previously described. Sensibility of the galvanometer was 1/50. The series of polarograms from C8 to A8, thus obtained,

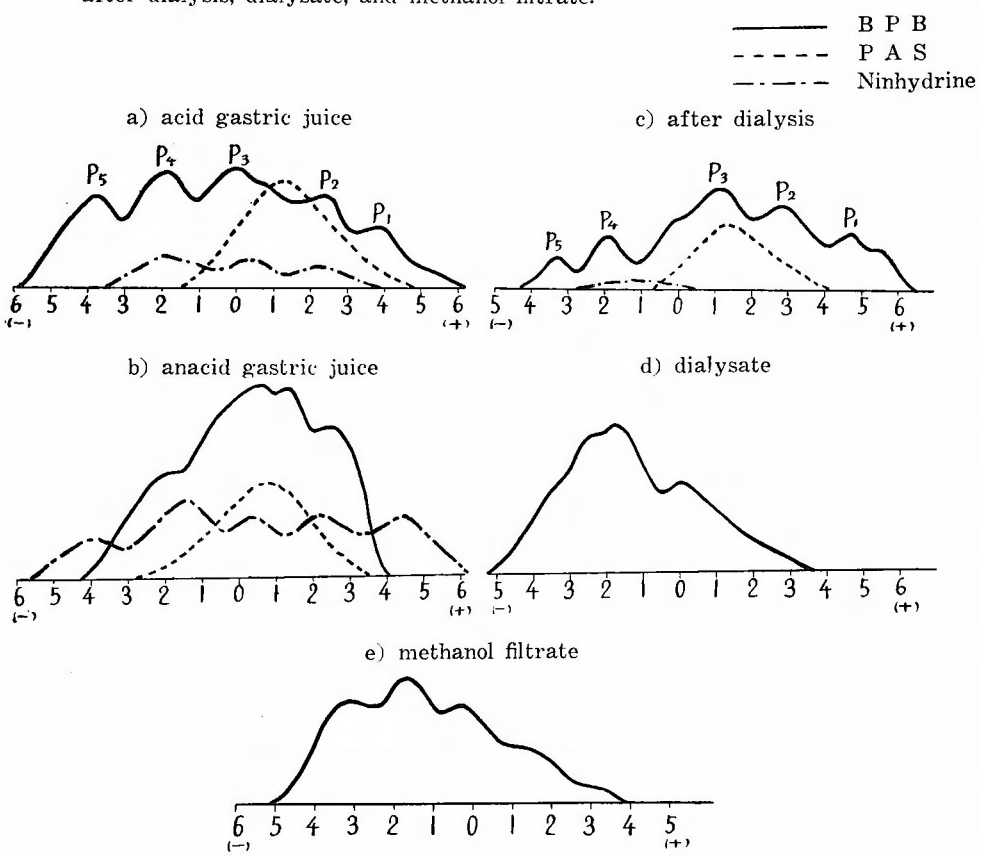
was called a paper electrophoresis-polarogram (abbreviated as PPgram).

RESULTS

1) Comparison between acid and anacid gastric juice

Materials A and B were used in this study. The average paper electrophoretic pattern of acid gastric juice as compared to that of anacid gastric juice is shown in Fig. 1 (a and b). With the BPB stain, 5 main peaks are found in the electrophoregram of the acid gastric juice. They are named P_1 to P_5, counting from the

Fig. 1 Comparison of electrophoregram of acid and anacid gastric juice before and after dialysis, dialysate, and methanol filtrate.

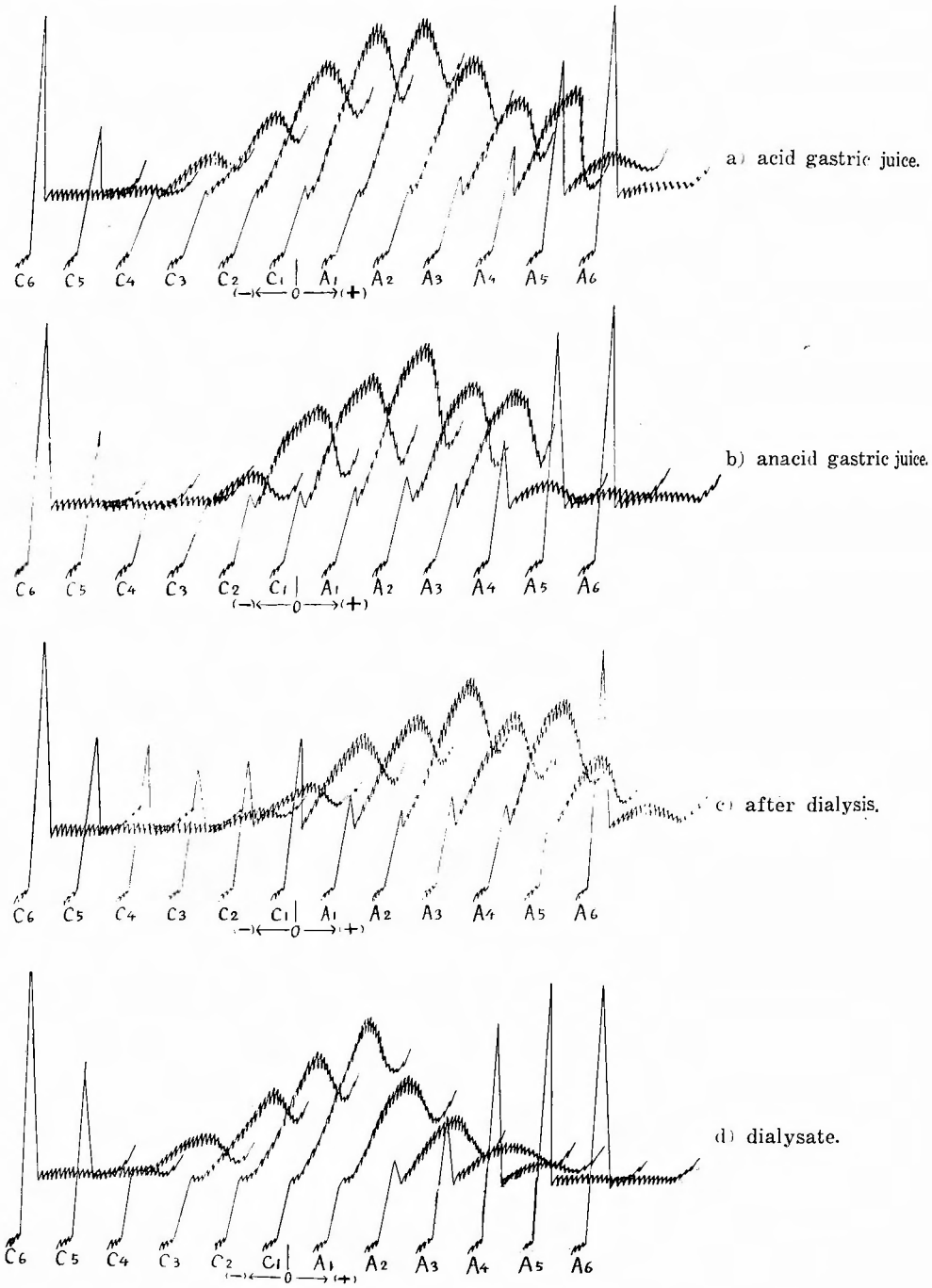


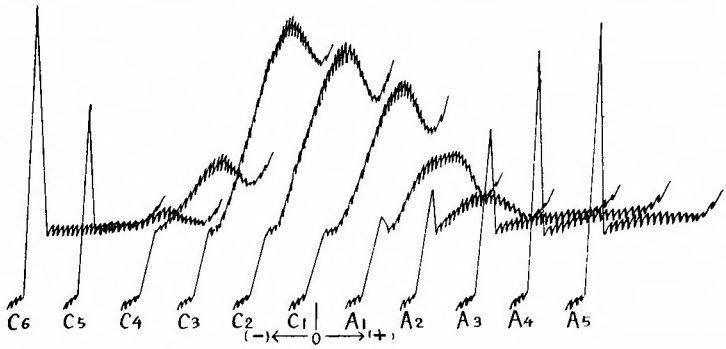
most anodic peak to the most cathodic peak. On the other hand, in the electrophoregram of anacid gastric juice 3 (P_2, P_3, P_4) out of 5 peaks are prominent, and the remaining 2 peaks, P_1 and P_5 are very low or absent. With the PAS stain only one difinite peak is found in the electrophoregrams of both acid and anacid gastric juice. The peak of the anacid juice is more prominent but its distribution is more narrowed on the anodic side than that of acid juice. With the ninhydrine stain, the peaks are more abundant and prominent in the electrophoregram of the anacid juice than that of the acid gastric juice.

The PPgrams of acid and anacid gastric juice are shown in Fig. 2 (a and b).

The PPgram of anacid juice is charaterized by very low, or absent protein waves in segments A_5, A_4, C_3 and C_4, in other words, their distribution is narrowed on

Fig. 2 Comparison of PPgram of acid and anacid gastric juice before and after dialysis, dialysate, and methanol filtlate.





both the anodic and cathodic sides and by the typical double wave with the low minimum point on both sides. The paper electrophoregram stained with BPB stain and the pattern of PPgram are nearly the same (as shown in Fig. 1). However, in the PPgram of the acid gastric juice protein waves have a wide range, from segment A_5 to C_1, with a single wave shape and markedly inhibited cobalt maximum on the cathodic side, and the typical double wave shape on the anodic side. Especially in segment A_1 the characteristic wave form is different from the the wave shapes of other segments.

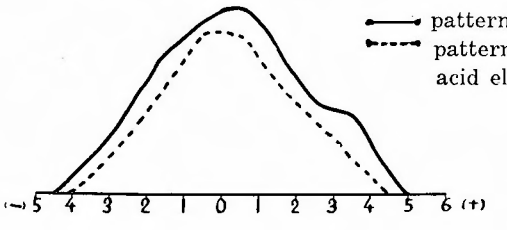
2) Influence of dialysis on electrophoregram and PPgram of acid gastric juice

Materials A, C and E were used in this study. The averaged paper electrophoregrams of acid gastric juice after dialysis are shown in Fig. 1 (c). With regard to the paper electrophoregram stained with BPB stain, all peaks are increased in sharpness but the height of P_5 and P_4 are decreased by dialysis. In the electrophoregram stained with PAS stain dialysis has little influence on the peak. With regard to the electrophoregram stained with ninhydrine stain peaks are markedly diminished or disappear after dialysis. The electrophoregrams of dialysate and methanol filtrate are shown in Fig. 1 (d and e). After dialysis the protein wave of segments A_4 and A_5 are slightly increased in height, but those of C_1, C_3 and C_2 are decreased or have disappeared, and show a more prominent cobalt mixiumum at C_5, C_4 and C_3. The PPgrams of the dialysate and methanol filtrate are similar (Fig. 2 d and e). Protein waves are present from C_4 to A_2 as single waves. This single wave is named the peptide wave.

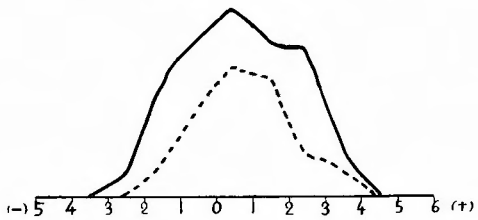
3) Protein precipitable by sulfosalicylic acid in PPgram

Fig. 3 Comparison of two PPgram obtained from 0.95% NaCl eluate and 10% sulfosalicylic acid eluate.

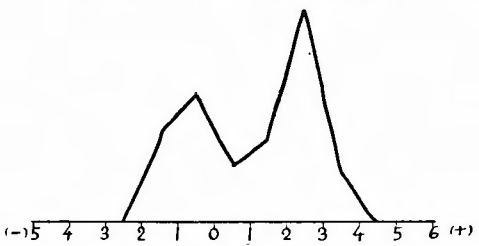
a) acid gastric juice.



b) anacid gastric juice.

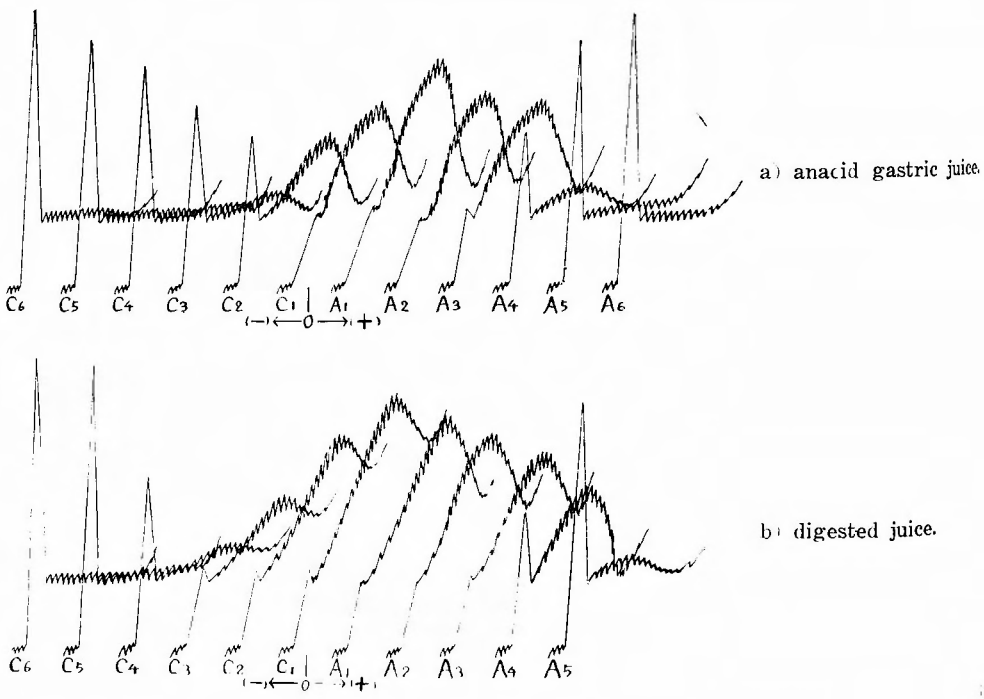


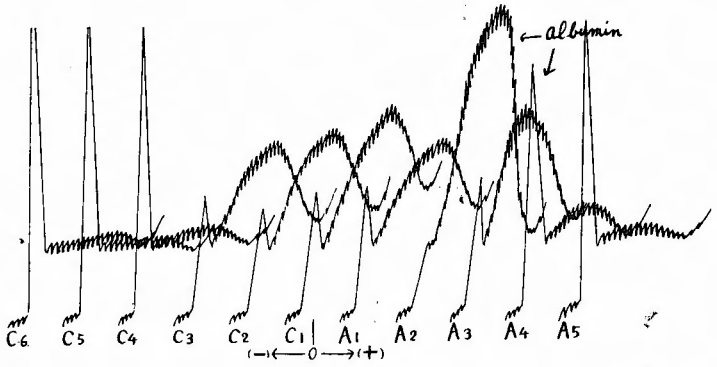
c) turbidity of electrophoretic fraction with sulfosalicylic acid added (anacid juice).



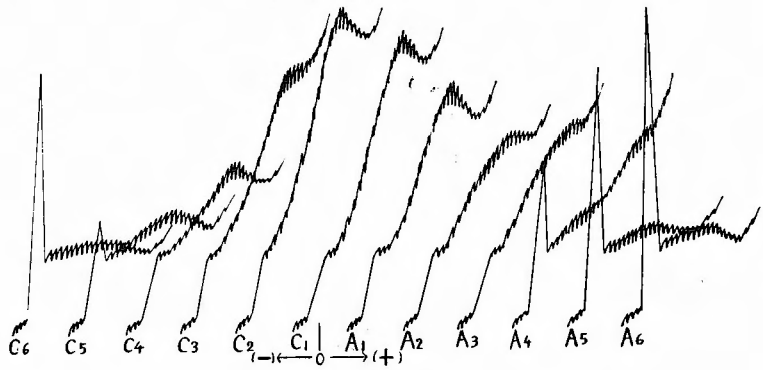
The pattern of PPgrams obtained from 10% sulfosalicylic acid and 0.95% NaCl eluates of acid and anacid juice are shown in Fig. 3. In acid gastric juice these two patterns are similar, but in anacid gastric juice the former is lower in segments A_3, A_2, A_1, and C_1 than the latter. The turbidity of 0.95% NaCl eluates with

Fig. 4 Changes in PPgram after pepsin digestion of anacid gastric juice and serum.





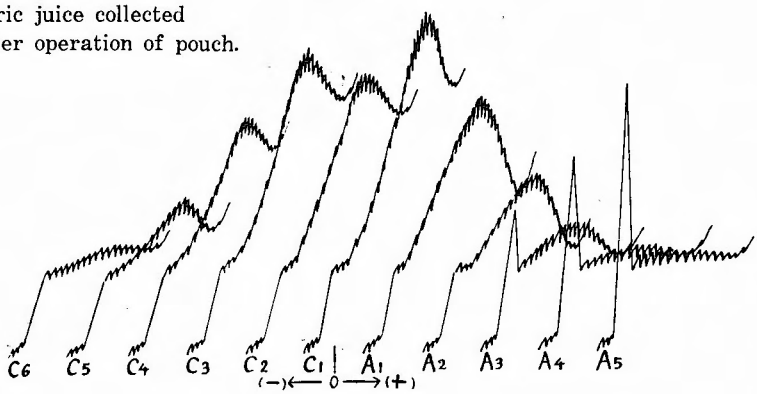
c) serum.



d) digested serum.

Fig. 5

a) canine gastric juice collected 2 weeks after operation of pouch.



b) canine gastric juice collected 4 weeks after operation of pouch.

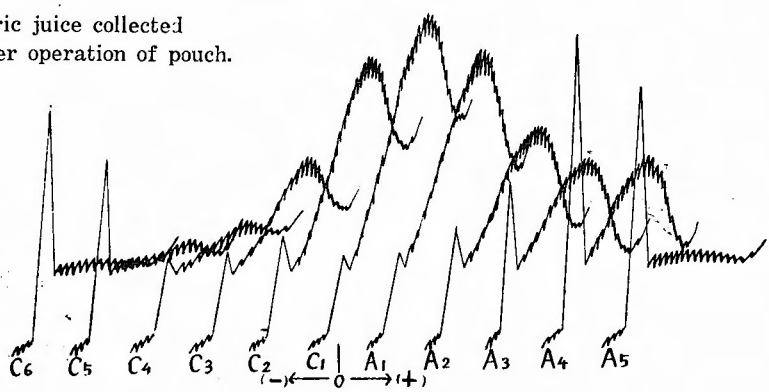
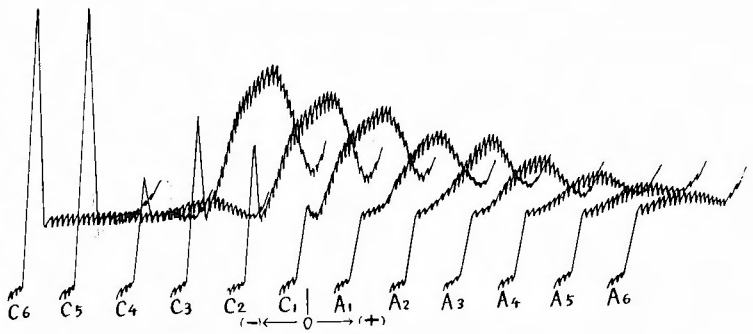


Fig. 6 PPgram of bile.



10% sulfosalicylic acid was scanned, traced and shown in Fig. 3 (c). Segment A_3 is the most turbid.

4) Influence of HCl-pepsin digestion on PPgram of anacid gastric juice and serum

Materials F, G and H were used in this study. The PPgram of the anacid juice specimen, serum, the pepsin digested anacid juice specimen and serum are shown in Fig. 4. In the PPgram of the digested gastric specimen the protein waves occur in a wide range from A_5 to C_4, as a single wave form on the cathodic side and as a double wave on the anodic side. In other words, PPgram of the pepsin digested anacid gastric specimen is similar to that of the acid juice specimen.

In the PPgram of native serum protein waves occur from A_3 to C_3 and have the typical double wave. The protein wave in segment A_2 and A_1 are the most prominent and characteristic, corresponding to albumin, and different from the wave corresponding to globulin.

In the PPgram of digested serum, protein waves have a wide range from A_4 to C_5 as typical single waves, or anomalous waves with a high minimum point.

5) Influence of operation on PPgrams of canine gastric juice

Material J was used. The PPgrams of gastric juice collected from pouch dogs 2 and 4 weeks after operation are shown in Fig. 5. In the PPgram taken 2 weeks after operation the peptide waves appear as far on the cathodic side as segment C_3. In the PPgram 4 weeks after operation the protein waves are found in an almost normal range.

6) PPgram of bile

This is shown in Fig. 6. The protein waves are found from A_3 to C_2 as the characteristic double wave with markedly inhibited cobalt maximum.

DISCUSSION

Electrophoretic studies on dialysed gastric juice protein have been reported by many investigators, but there have been few on nondialysed gastric juice protein.

In the previous report[10] it was demonstrated that a detectable amount of peptide was consistently present in acid gastric juice, and this peptide was soluble in methanol and was dialysable through a cellophane membrane. The peptide in

the methanol filtrate and dialysate have the same cathodic electrophoretic mobility in a borate buffer of pH 9.0 and the same polarographic single wave as shown in Fig. 1 and 2. It seemed that both peptides are almost identical.

The difference between the PPgram with and without dialysis as shown in Fig. 2 also indicates that a considerable amount of dialysable peptide is present in acid gastric juice.

The protein of anacid juice is eletrophoretically in narrow range both on the cathodic and anodic side from A_3 to C_1, and polarographically characterized by a typical double wave. However, the protein of acid juice is electrophoretically in a wide range from A_5 to C_1, and polarographically characterized on the cathodic side by a single wave, which is similar to the wave of pepsin digested serum, and by atypical double wave on the anodic side. Particularly the protein wave in segment A_4 shows a charateristic double wave with a very low minimum point. Because this segment A_4 corresponds to segment M_1 of GLASS,[17] this protein wave is probably produced by the mucoprotein of GLASS. This protein wave is named the mucoprotein wave. The mucoprotein wave is more prominent and typical in the PPgram of acid juice after dialysis, and is not found in the PPgram of anacid juice. When anacid gastric juice is digested by HCl-pepsin, the mucoprotein-like wave is found in the same segment A_4. From these data the possibility cannot be excluded that mucoprotein is also the pepsin digestive product.

It is evident that the protein of anacid gastric juice has an electrophoretically and polarographically different nature from the protein of acid gastric juice. It may be emphasized that the anacid pattern is changed to the acid pattern by pepsin digestion just as the native serum pattern is changed to the peptide pattern. GLASS [18] has reported that the electrophoregram of the gastric jucie of patients with gastric atrophy is characterized by the complete absence of pepsin, M_1, Y and Z peaks and an absence of, or a very low, X peak. These characteristics of gastric atrophy are always found as the pattern of anacid juice. It is more reasonable for the characteristic pattern of gastric juice of patients with gastric atrophy to be called anacid pattern not atrophic pattern, although achlorhydria and gastric atrophy are intimately related. In other words, the gastric juice must always be anacid for the atrophic pattern to appear.

In anacid gastric juice protein precipitable by sulfosalicylic acid is consistently present as described previously.[10] This protein is electrophoretically distributed from A_3 to C_1, especially in segment A_3, like serum protein.

GLASS[19] reported that soluble mucus was present in filtered gastric juice and corresponded to the middle peaks of the electrophoretic pattern of whole gastric juice, and was precipitated with trichloracetic acid. The protein precipitable by sulfosalicylic acid may be identical with GLASS's soluble mucus. It is interesting that HCl-pepsin digestion causes this protein in anacid juice to disappear, whereas peptide and mucoprotein-like protein appear.

With regard to dialysed acid gastric juice, the protein waves in segments C_2, C_3, C_4 and C_5 presumably correspond to the X, Y, Z components of GLASS.[17] He

reported that the X component contained almost no carbohydrate, only a small amount of protein and much nondialysable material unidentified as yet. The protein waves in segments C_1 and C_3 are much lower than would appear from the densitometric tracing stained with BPB stain. These data indicate that the protein waves in segments C_2 and C_4 correspond to the X component of GLASS. The Y, and Z components are the most positively charged cathodic components, and contain no protein or carbohydrates at all according to GLASS. The protein waves in segments C_4, C_5 are very low or absent and are characterized by the inhibited cobalt maximum, although height of the cobalt maximum is increased to some extent after dialysis. This indicates that at segment C_4, C_5 cobalt maximum inhibiting high molecular substances are present. In the PPgram of bile the cobalt maximum of the protein wave is markedly inhibited, especially on the anodic side. Inhidition of the cobalt maximum is closely related to surface active agents in general. The substance inhibiting the cobalt maximum in bile is presumably bile pigment, because bile pigment has anodic electrophoretic mobility. In the PPgram of gastric juice obtained from pouch dogs 2 weeks after operation the protein waves appear widely on the cathodic side as far as segment C_2. It seems likely that an increased amount of protein, which is secreted from the mucosa of the post-operative gastritis, is digested by pepsin and broken down to peptide. BALLE-HELAERS[20] and SASAI[21] have reported that the first maximum of a protein double wave originates in protein-bound polysaccharides in general. Indeed, in the area showing the typical double wave polysaccharide is always present in the PAS stain. On the contrary, in areas showing a single wave polysaccharide can not be found by PAS stain. Therefore the peptide in methanol filtrate or dialysate may not be conjugated with polysaccharide. The single wave with high minimum point is also found in serum albumin[22][23] and ovoalbumin[24][25] denatured by alkali or heat.

The characteristics of wave shape may be studied as an important approach to the problem of the structure of protein molecules.

II. CLINICAL OBSERVATION

On the basis of the experimental results described above, clincal studies were carried out with special emphasis on cancer of the stomach.

MATERIALS

Studies were carried out on 59 subjects with the following diseases: Gastric cancer 19, precancerous state 2, peptic ulcer 7, duodenal ulcer 8, aplastic anemia 3, other diseases with acid gastric juice 13 (gastric ptosis, chronic gastritis, cholelithiasis), gastric polyp 1, leiomyoma of the stomach 1, other diseases with anacid gastric juice, 5. All cases with the exception of 3 with aplastic anemia were operated upon, and the final diagnosis was confirmed in all instances by histologic examination of the resected specimen in the Pathological Laboratory of the Kyoto University Hospital.

METHODS

1) Aspiration of gastric juice

After fasting for about 12 hours, gastric juice was aspirated through a REHFUSS stomach tube, followed by caffeine stimulation (KATCH-KALK method), or sometimes by histamine stimulation. During the aspiration of the specimens, the greatest possible care was taken to avoid contaminations with saliva, blood or bile. Specimens apparently contaminated with bile, blood or food were not used. The specimens were subsequently filtered through filter paper (Toyo Roshi No. 5c).

2) The free acidity was titrated by using as indicator TOPFER's reagent.

3) Polarograms were taken on 3 fractions: buffered fraction (Fb), sulfosalicylic filtrate fraction (Fs) methanol filtrate fraction (Fm) as described previously.[10]

4) Paper electrophoregrams of BPB stain, PAS stain and ninhydrine stain, and PPgrams were made on each of the nondialysed lyophilized gastric juice specimens as described above.

5) Paper electrophoregrams of BPB stain were divided into 5 areas corresponding to the 5 main peaks. These areas were termed B_1, B_2, B_3, B_4 and B_5, counting from +5cm to +3cm, from +3cm to +1cm, from +1cm to −1cm, from −1cm to −3cm, and from −3cm to −5cm, respectively, and each area was measured by a planimeter. With regard to the PPgram the wave height of each area was calculated as follows: the wave height of B_1, B_2, B_3, B_4 and B_5 was repesented by the wave height of segment A_4, averaged wave height of two segments A_3 and A_2, A_1 and C_1, C_2 and C_3, and the wave height of sement C_4, respectively.

Table 1

No.	Name	Free acidity	Electrophoregram					PPgram (%)					Fb Value	Fm Value	Fs Value
			B_1	B_2	B_3	B_4	B_5	B_1	B_2	B_3	B_4	B_5			
acid gastric cancer															
273	Urushizaki	hypo	2	17	29	28	24	11	27	33	18	11	32	30	30
291	Shiozaki	normo	9	22	29	23	17	15	29	33	19	4	47	44	47
303	Takahashi	hypo	3	18	32	27	20	6	24	45	20	5	24	21	24
310	Higashihara	normo	11	27	24	29	9	20	33	34	11	2	46	40	46
327	Iwai	hypo	7	19	19	25	30	13	27	40	15	5	26	24	24
343	Hayashi	hypo	10	13	26	27	24	23	36	31	12	3	21	15	18
353	Hatabayashi	hypo	22	24	30	14	10	20	39	30	10	1	24	17	19
366	Tanaka	hypo	4	15	21	31	29	9	27	39	18	7	26	21	24
369	Matunaga	hypo	12	31	27	18	12	18	30	35	9	2	23	17	21
270	Katō	normo	9	22	26	25	18	14	39	32	15	0	29	28	29
average value			9	21	26	25	19	15	31	35	15	4	30	26	28
anacid gastric cancer															
247	Usui	anacid	4	23	50	23	0	0	29	45	16	10	18	7	7
284	Baba	anacid	0	15	40	40	5	0	40	35	16	9	29	15	17
292	Nishi	anacid	7	40	42	11	0	9	41	35	15	0	40	22	23
306	Tanino	anacid	5	31	30	31	3	10	32	33	24	1	37	20	20
324	Mori	anacid	6	30	22	36	6	9	55	23	13	0	23	6	11
333	Ueyanagi	anacid	2	34	31	30	3	6	35	40	18	1	15	5	5
348	Yamagata	anacid	11	32	28	27	2	10	40	30	18	2	20	8	9
350	Akamatsu	anacid	5	31	47	17	0	0	37	44	19	0	22	12	10
354	Kinoshita	anacid	6	15	28	32	19	10	38	35	16	1	11	4	4
average value			5	29	35	27	4	6	39	36	17	2	24	11	12

precancerous states

338	Mise	hypo	10	22	27	27	14	10	23	43	20	4	37	31	36
361	Kawasima	hypo	9	21	32	26	12	15	28	35	17	5	27	25	27
average value			10	22	29	26	13	13	26	39	18	4	32	28	32

peptic ulcer

304	Oka	hyper	14	20	35	24	7	17	28	32	18	5	19	13	18
311	Nakazima	normo	11	18	24	27	20	10	35	37	13	5	25	23	25
323	Ukita	hyper	21	27	23	18	11	19	26	41	10	4	13	10	13
325	Murata	hyper	15	15	21	27	22	15	20	30	27	8	16	13	14
339	Ōdati	hyper	15	19	27	23	16	22	21	33	21	3	15	9	10
352	Kimura	hyper	11	20	25	30	14	19	26	36	14	5	30	24	31
362	Yokota	hypo	15	24	24	28	9	14	33	40	11	2	10	8	9
average value			15	20	25	25	15	17	27	35	16	5	18	14	17

duodenal ulcer

301	Ōkuwa	hyper	14	24	30	19	13	16	29	31	20	4	22	18	21
309	Matsutani	hypo	12	19	22	29	18	21	31	33	9	6	12	8	11
328	Yamasaki	hyper	14	18	26	22	20	22	23	33	17	5	16	10	14
330	Minami	hyper	21	22	22	23	12	24	26	35	12	3	16	12	16
336	Fukui	hyper	21	18	27	17	17	15	31	33	20	1	30	19	29
340	Yasui	hyper	11	19	33	20	17	14	30	39	14	3	14	7	13
363	Yamamoto	hyper	15	18	31	25	11	18	34	34	12	2	17	11	15
371	Ebara	hyper	13	14	25	39	9	23	30	29	16	2	8	8	8
average value			15	19	27	24	15	19	29	33	15	3	17	12	16

aplastic anemia

335	Miyosi	hyper	16	20	30	24	10	20	17	30	25	8	18	13	18
337	Imai	normo	20	19	22	24	15	24	22	30	18	16	13	8	13
359	Yamamoto	hyper	16	20	24	22	18	16	30	40	12	2	14	8	13
average value			17	20	25	23	15	20	25	34	28	13	15	10	15

Others (acid)

345	Nakatani	hyper	16	22	28	20	14	17	33	34	12	4	14	9	13
346	Funagoshi	hyper	17	21	22	25	15	19	24	32	20	5	27	15	27
351	Nakae	hyper	10	19	28	27	16	15	29	32	20	4	25	17	25
365	Suzumura	hyper	10	17	29	30	14	13	31	39	15	2	20	12	20
307	Hatano	normo	17	18	23	22	20	12	27	36	18	7	34	26	30
322	Masuda	hyper	15	22	26	24	13	17	30	34	15	4	16	8	16
347	Nishigami	hyper	8	24	33	28	7	15	36	37	9	3	7	6	7
360	Takarada	normo	16	20	26	25	13	23	32	33	9	3	30	22	30
370	Wakabayashi	normo	11	18	25	31	15	10	35	38	15	2	24	20	23
232	Asai	hyper	15	19	26	25	15	16	29	35	16	4	17	14	17
220	Hiranabe	normo	16	20	27	25	12	18	28	33	17	4	21	16	20
391	Izumiyama	normo	10	21	27	30	9	20	31	33	13	3	16	12	16
380	Ito	normo	17	21	24	27	11	17	28	33	18	4	19	14	18
average value			14	20	27	26	13	16	30	35	15	4	21	15	20

gastric polyp

266	Tanaka	anacid	1	40	44	14	1	4	43	37	16	0	22	13	14

Leiomyoma of the stomach

270	Hōki	anacid	7	42	37	14	0	15	46	31	8	0	31	9	19

Others (anacid)

329	Murata	anacid	0	30	59	11	0	0	44	44	12	0	6	3	6
356	Hosoki	anacid	3	24	43	27	3	7	52	33	8	0	5	4	4
367	Mizutani	anacid	11	37	27	25	0	5	34	50	11	0	6	5	6
358	Kaimoto	anacid	6	27	43	23	1	4	24	52	20	0	4	3	3
373	Kanda	anacid	5	34	39	22	0	6	40	42	12	0	9	4	4
380	Ito	anacid	5	35	42	18	0	10	43	32	15	0	16	7	8
average value			5	31	42	21	1	5	39	42	13	0	9	4	5
non-cancer (acid) average			15	20	26	25	14	18	28	34	16	4			

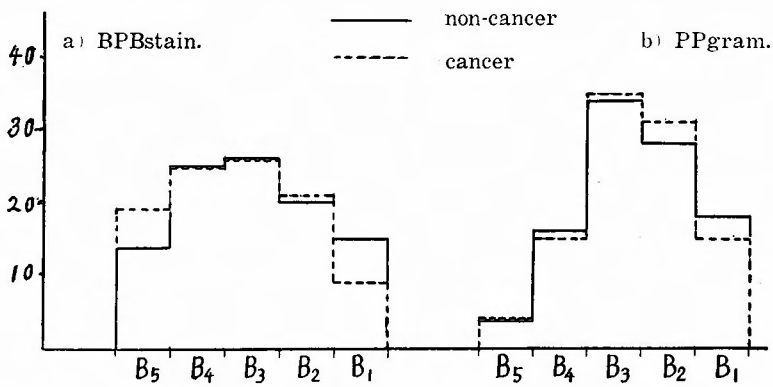
RESULTS

Detailed data of the polarograms of 3 fractions (Fb, Fm, Fs), the electrophoregram of BPB stain, and the PPgram of 59 cases are presented in Table 1. No. 380 is a patient with caffeine refractory anacid juice, but with acid juice following histamine stimulation.

1) Comparison between gastric cancer group and non-cancer group with acid gastric juice

The average electrophoregrams and PPgrams of both groups are shown in Fig. 7.

Fig. 7 Comparison between gastric cancer and non-cancer cases with acid gastric juice.



BPB stain: In Fig. 7 a the BPB staining area of B_1 of the cancer group is smaller than that of the non-cancer group. On the contrary, the area of B_5 of the cancer group is larger than that of the noncancer group. The remaining areas of both groups are almost identical.

PPgram: In Fig. 7b there is very little difference between the two groups in wave height (area), but a marked difference in wave form. The typical mucoprotein wave (segment A_1) is found in only 2 out of 10 cancer cases but is found in all except 2 noncancer cases, being especially prominent in cases of duodenal ulcer. The peptide wave (on cathodic side) of gastric cancer is charcterized by a high minimum point against the wave height, and by markedly inhibited cobalt maximum. The

Fig. 8 Proportion of height of minimum point to protein wave height at segment C_2

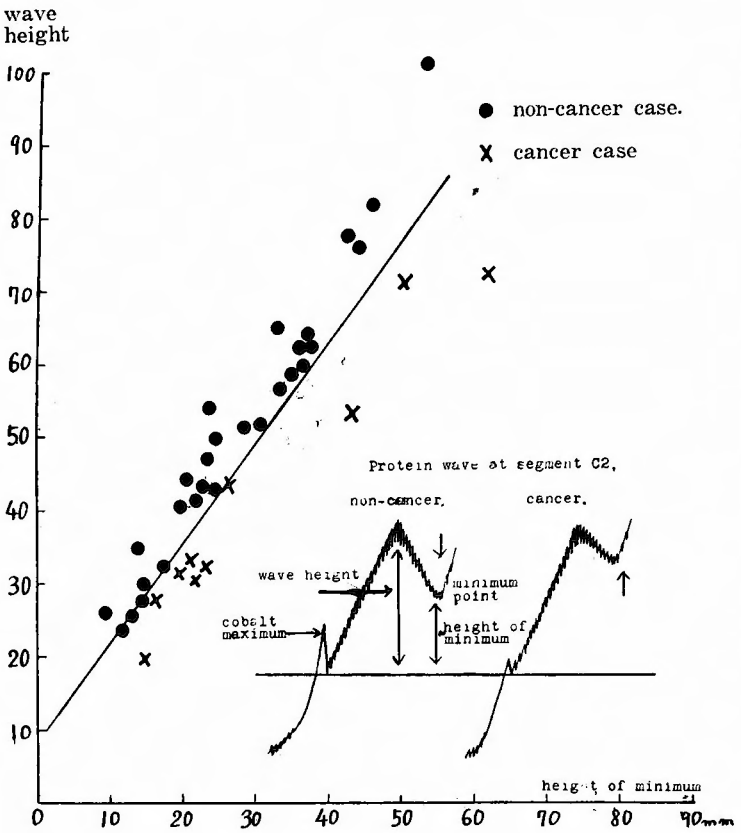


Fig. 9 Comparison of cancer and noncancer in height of cobalt maximum at C_5

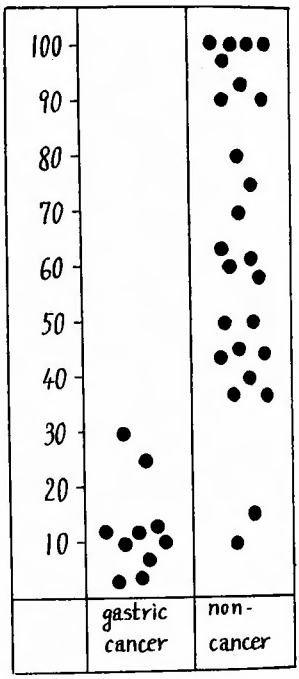
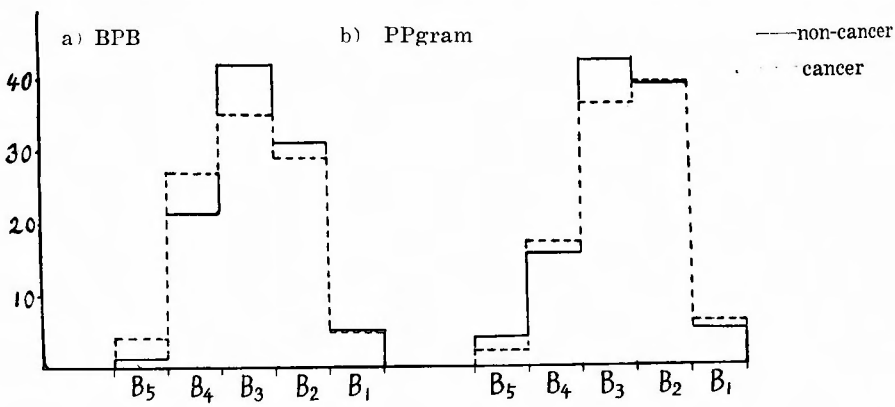
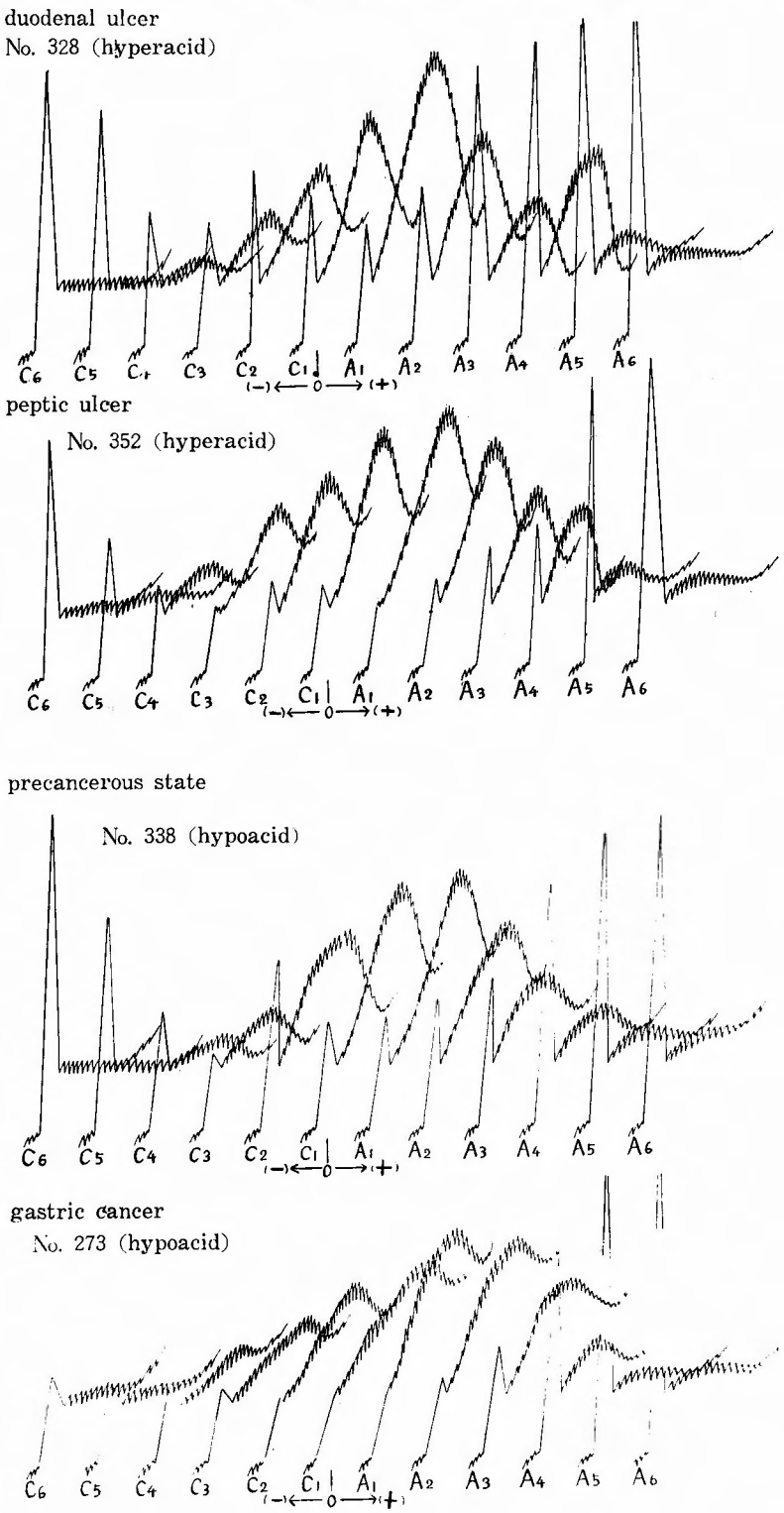


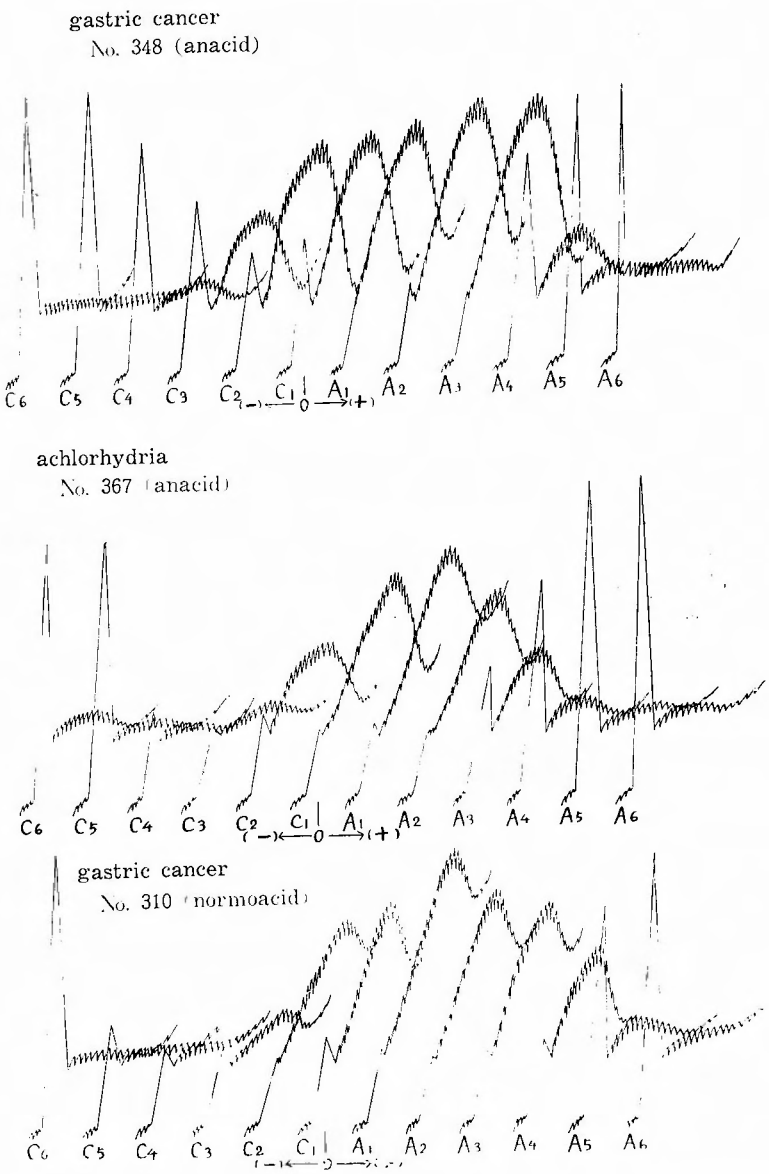
Fig. 10 Comparison between gastric cancer and non-cancer cases with anacid gastrc juice.



proportion of the height of the minimum point to the wave height in segment C_2 and a typical peptide wave of cancer and non-cancer cases are shown in Fig. 8. The two groups are compared in terms of the height of the cobalt maximum at segment C_5 as shown in Fig. 9. Moreover, the cases characterized by a high min-

Fig. 11 PPgrams of cancer and non-cancer





imum point indicate a high Fm value on fractional polarographic analysis.

2) Comparison between both groups with anacid juice

The 2 groups were compared in terms of the average electrophoregram of BPB stain and the PPgram as shown in Fig. 10. There is little difference between the 2 groups in regard to the electrophoregram and PPgram which are characterized by a narrow range of A_3 and C. The protein waves in the PPgram are typical double waves. In a few cancer cases, anomalous waves appear at segments A_5 and A. These anomalous waves disappear after dialysis.

Typical PPgrams of cancer and non-cancer cases are shown in Fig. 11.

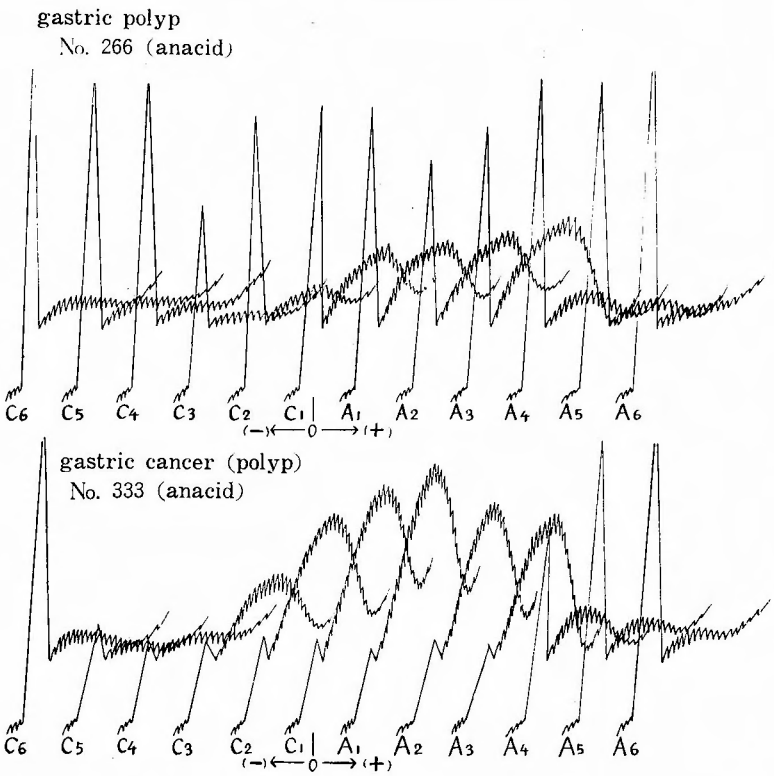
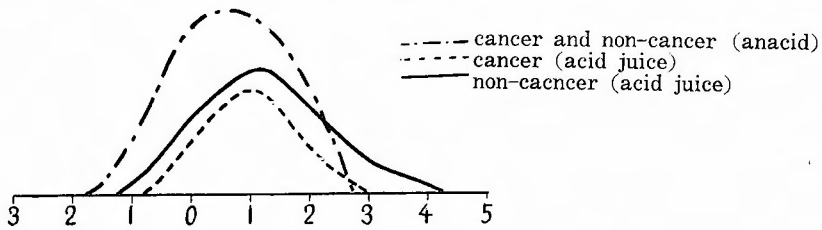


Fig. 12 Electrophoregram of PAS stain



3) Electrophoregram of PAS stain

The two groups are compared in terms of the average electrophoregram of PAS stain as shown in Fig. 12. In the anacid gastric juice there is scarcely any difference between the two groups, but in acid gastric juice there is a difference on the anodic side as shown in Fig. 12.

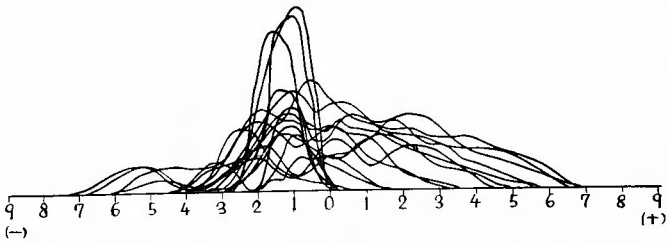
4) Electrophoregram of ninhydrine stain.

In the electrophoregrams of cancer cases the peaks are more numerous and prominent than in non-cancer cases as shown in Fig. 13. These peaks are diminished or disappear after dialysis.

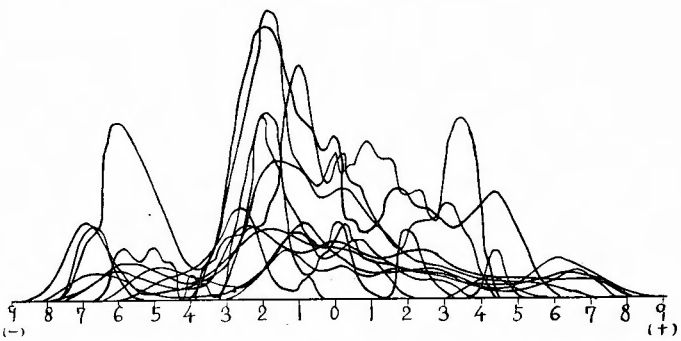
DISCUSSION

Although the electrophoretic pattern of gastric juice is variable as has been

Fig. 13 Electrophoregram of ninhydrine stain.
a) non-cancer cases.



b) cancer cases.



reported by many investigators,[1)~9),23)] 5 main components are generally found in the author's data.

The component (B_4) of the most anodic electrophretic mobility, which corresponds to the component M_1 of GLASS,[17)] is increased in acid non-cancer juice, especially in duodenal ulcer, decreased in acid gastric cancer juice, and absent in all anacid cases. These data agree with the data of other investigators.[1),3),8),9)] This component is polarographically represented as the characteristic protein wave which is named the mucoprotein wave. The mucoprotein wave is typical in acid non-cancer juice, especially prominent in duodenal ulcer, low or atypical in acid gastric cancer and not present in achlorhydria. It is interesting that the rise and fall of the mucoprotein wave parallels that of the glandular mucoprotein obtained by the acetone precipitation method of GLASS.[27)] The mucoprotein wave may be related to the intrinsic facor of CASTLE, because glandular mucoprotein serves as the intrinsic factor according to GLASS.[28)] Although NORPOTH[1)] and GROSSBERG[1)] have reported that the fast anodic moving component represents the same enzymatic action as pepsin, the mucoprotein wave evidently differs from the protein wave of pepsin itself.

The component (B_4) of acid gastric cancer stains only slightly with PAS stain. The reason for this difference may be that glandular mucoprotein is decreased in acid cancer.

The component (B_5) of the fastest cathodic electrophoretic mobility, which

includes components Y and Z of GLASS,[17] is increased in acid gastric cancer, and absent in achlorhydria, and does not stain with PAS stain. This component is characterized by a low cobalt maximum in the PPgram. The protein wave of this component is much lower than would appear from the densitometric tracing stained with BPB stain, especially in acid garstric cancer. The cobalt maximum is markedly inhibited in acid gastric cancer. It indicates that there is a large amount of cobalt maximum inhibiting factor and a low polarographic-active substance in the gastric juice of patients with gastric cancer. The author has recenly obtained some evidence that the toxohormone-peptide may be located in this component, but this is still under investigation.

The slow cathodic moving component polarographically represents a single wave which is named the peptide wave in the acid cases, and a typical double wave in the anacid cases. There is a difference between acid cancer and non-cancer in respect to the form of the peptide wave. Gastric cancer is characterized by a peptide wave with a relatively high minimum point to wave height ratio as shown in Fig. 8. It is unexpected that there is no significant difference between cancer and non-cancer group with regard to wave height of B and B_5. The case characterized by a protein wave with a high minimum point in the PPgram represents a high protein value in the methanol filtrate (Fm). This indicates that there is a large amount of peptide in acid gastric juice obtained from gastric cancer.

It is evident that the low or atypical mucoprotein wave and the peptide wave with high minimum point and markedly inhibited cobalt maximum can be used as factors in the diagnosis of gastric cancer, if the gastric juice is acid.

The distribution of proteins in the eletrophoretic pattern of anacid gastric juice appears similar when polarography is applied to the eluates of segments cut from paper electrophoretic strips. The electrophoretic distribution of the proteins of anacid gastric juice is in a narrow range both on the anodic and cathodic side. These data agree with the data of other investigators.[3),8),9),18),19)] The protein waves when present are always found as typical double waves in the PPgram. There is no difference between anacid cancer and non-cancer in respect to the electrophoregram of BPB stain and PAS stain, and PPgram. In consequence the differencial diagnosis between anacid cancer and non-cancer is impossible. The incidence of atrophic gastritis is too low to discuss.

The slow anodic moving components (B_2 and B_3), which presumably correspond to the mucoproteose of GLASS,[17] are large in anacid cases. These data agree with the data of other investigators.[3),8),9),18),19)] The protein waves of these components are found as typical double waves in the anacid cases, and as atypical double waves with a high minimum point in the acid cases. Because the protein precipitable by sulfosalicylic acid is distributed in the same area as described above, the typical double wave appears in this area in anacid cases.

It is evident from the protein wave form that the components of identical electrophoretic mobility is not always the same.

In the electrophoregram of the ninhydrine stain, the peaks are abundant and

prominent in anacid gastric cancer. These peaks are diminished or disappeared after dialysis. The author has recently proved by using high voltage paper electrophoresis and polarography that ninhydrine stained substances are amino acid and dialysable low molecular peptide.[29] It is evident that a large amount of amino acid is present in the gastric juice of patients with gastric cancer. These data agree with OHUCHI et al[30] and GILLIGAN'S[31] data obtained by using paper chromatography.

SUMMARY

1) Five main components were found in the electrophoretic pattern of gastric juice.

2) The peptide in the methanol filtrate and dialysate was distributed mainly on the cathodic side, polarographically represented as a single wave (peptide wave) and did not stain with PAS stain.

3) There is a significant difference between acid and anacid cases in the electrophoregram and PPgram. In the electrophoregram of acid cases there is a wide distribution. In the anacid cases the distribution is narrow. The PPgram of the acid cases was characterized by a single wave on the cathodic side, by an atypical double wave on anodic side and by a mucoprotein wave on the most anodic side, that of the anacid cases was characterized by a typical double wave.

4) The PPgram of the anacid cases was changed by pepsin digestion to resemble the PPgram of acid cases.

5) Albumin was polarographically represented by a high protein wave with a low minimum point differing from a globulin wave. The PPgram of serum was changed by pepsin digestion to the peptide pattern.

6) The mucoprotein wave was typical and prominent in acid non-cancer, atypical or low in acid cancer and not present in anacid caces.

7) The peptide wave of acid cancer was characterized by a high minimum point.

8) The cobalt maximum on the cathodic side was markedly inhibited in acid cancer.

9) The amount of amino acid was increased in cancer espedially in anacid cancer.

ACKNOWLEDGEMENT

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和文抄録

胃液内蛋白の研究

第2報 胃液内蛋白の濾紙電気泳動－ポーラログラフ的研究

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筧 守

凍結乾燥した胃液について濾紙電気泳動を行い，その濾紙を1糎巾に切つて溶出しポーラログラフで蛋白波を撮つて検べる新しい方法(PPgram)を用いて，胃疾患特に胃癌の胃液内蛋白像の変化を検索して次の結果を得た．

1) 胃液蛋白は電気泳動上5つの分画に分れる．

2) メタノール濾液及び透析外液内のPeptideは泳動上主として陰極側に移動し，PAS染色では染らず，ポーラログラフでは一重波を示す．

3) 有酸胃液と無酸胃液とでは泳動図上でも，PPgram上でも明らかに異つたPatternを示す．即ち有酸では広い泳動図を，無酸では狭い泳動図を示し，またPPgramでは，有酸では陰極側で一重波を陽極側では二重波を示し，特に最も陽極側は特異な蛋白波(Mucoprotein Wave)を示した．一方無酸では総て二重波を示した．

4) Mucoprotein Waveは有酸非癌例で高く，特に十二指腸潰瘍例で典型的且つ著明で，胃癌例は低いか非典型的であり，無酸例は癌，非癌をとわずMucoprotein Waveは見られなかつた．

5) 無酸のPPgramは，無酸胃液蛋白をPepsinで消化することによつて有酸のPPgramに変えることが出来た．

6) 有酸の胃癌例では陰極側のPeptide Waveは Minimum Pointが高く，コバルト極大も特に強く抑制されるのが特異である．

7) 胃癌胃液内にはアミノ酸の増量を認めた．

以上の結果から，胃癌胃液のPPgram上の特長は鑑別診断上意義あるものと考えられる．